Food Chemistry 239 (2018) 733–741

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Phenolic acids, anthocyanins, proanthocyanidins, antioxidant activity, minerals and their correlations in non-pigmented, red, and black rice

Yafang Shao [a], Zhanqiang Hu [a], Yonghong Yu [a], Renxiang Mou [a], Zhiwei Zhu [a,*], Trust Beta [b,*]

[a] China National Rice Research Institute, Hangzhou 310006, China
[b] University of Manitoba, Department of Food and Human Nutritional Sciences, Winnipeg R3T 2N2, Canada

ARTICLE INFO

Article history:
Received 23 May 2017
Received in revised form 20 June 2017
Accepted 2 July 2017
Available online 3 July 2017

Keywords:
Whole rice grain
Phenolic acids
Anthocyanins
Proanthocyanidins
Antioxidant activity
Minerals
Principal component analysis

ABSTRACT

Soluble-free, soluble-conjugated, insoluble-bound phenolics and antioxidant activity, flavonoid (TFC), proanthocyanidins (TPAC), anthocyanins and minerals of fifteen whole rice grains with different colors were investigated. Soluble-free protocatechuic and vanillic acids were only quantified in black rice, which had the most quantities. Non-pigmented rice had no detectable conjugated protocatechuic and *2,5*-dihydroxybenzoic acids both of which were found in black and red rice, respectively. The main bound phenolic acids were ferulic and *p*-coumaric, as well as *2,5*-dihydroxybenzoic in red rice and protocatechuic and vanillic acids in black rice. Soluble-conjugated phenolics, TFC, and anthocyanins were negatively correlated with L^*, b^*, C and H° values. TPAC was positively correlated with a^* ($P < 0.01$). Protocatechuic, vanillic, syringic and ferulic acids were associated with TPC and antioxidant activity in the soluble-conjugated fraction while protocatechuic and ferulic acid were correlated with those in the insoluble-bound fraction. Principal component analysis divided samples into non-pigmented, red and black rice groups.

1. Introduction

Rice (*Oryza sativa* L.), as one of the most important staple crops, provides energy for almost half of the world's population. Because of the tradition of eating milled or polished rice, the monotonous consumption of rice may lead to deficiencies of essential vitamins, minerals and other nutritional and functional compounds (Bouis, Chassy, & Ochanda, 2003). Most of the phytochemicals are present in bran layer and embryo fraction (Shao, Xu, Sun, Bao, & Beta, 2014a). Whole rice grains are hypothesized to contribute positively to human health due to their polyphenols, minerals, fibre, vitamins and other phytochemicals (Deng, Xu, Guo, Xia, & Li, 2012; Deng et al., 2013; Min, Mcclung, & Chen, 2011). These compounds may influence biological functions individually or synergistically. Many interventional and epidemiological studies show that the consumption of whole rice grain is associated with the reduction in risk of developing chronic diseases such as cardiovascular diseases, obesity, type II diabetes and some cancers (Mattei et al., 2015; Shao & Bao, 2015).

Phenolics, as a large group of secondary metabolites in cereal grains, are present in three forms, soluble-free, soluble-conjugated and insoluble-bound (Li, Shewry, & Ward, 2008; Park et al., 2012). The soluble phenolic acids would become absorbed in the stomach and small intestine for distribution to the whole body with concomitant health benefits such as inhibition against oxidation of low-density lipoprotein cholesterol and liposomes (Min, Gu, Mcclung, Bergman, & Chen, 2012; Shao & Bao, 2015), while the insoluble bound phenolic acids would be accessible in the intestine after digestion by the enzymes and some are released in the colon by the colonic microflora (Saura-Calixto, Serrano, & Goñi, 2007). Microelements (Zn, Mn, Cu and Fe) and macroelements (P, K, Ca and Mg) can be found in every cell where they play important roles in maintaining normal metabolic functions, proper fluid balance, blood pressure regulation, nerve transmission, and immune system health (Speich, Pineau, & Ballereau, 2001).

Color is an important feature used by consumers for selection of food products. The pigments of black and red rice samples are due to the accumulation of anthocyanins and proanthocyanidins in rice bran, respectively (Min et al., 2011). The biosynthesis pathway of phenolic acids, anthocyanins and proanthocyanidins begins with L-phenylalanine via the phenylpropanoid pathway (Shao & Bao, 2015). Using classical genetic analysis, two loci that are associated with red pericarp have been identified (Sweeney, Thomson, Pfeil, & McCouch, 2006), and the regulation mechanisms of anthocyanin biosynthesis have been revealed (Oikawa et al., 2015). However,

* Corresponding authors.
E-mail addresses: zwzhu80@126.com (Z. Zhu), Trust.Beta@umanitoba.ca (T. Beta).

http://dx.doi.org/10.1016/j.foodchem.2017.07.009
0308-8146/

the biosynthesis regulatory relationships between phenolic acids and anthocyanins in different colored rice grains are largely unknown. Although the correlations among color parameters of L^*, a^*, b^*, C, $H°$, total phenolic, flavonoid content, and antioxidant activity were studied (Shen, Jin, Xiao, Lu, & Bao, 2009), the exact phenolic compounds that contributed to the color differences, total phenolic content, and antioxidant activity have not been identified.

In this study, we aimed to evaluate soluble-free, soluble-conjugated, and insoluble-bound phenolics, antioxidant activity, total flavonoids, proanthocyanidins, anthocyanins, and minerals of whole rice grains so as to determine their relationships with grain color, as well as the relationships between the specific phenolic acids and total phenolic content or antioxidant activity. The results of this study could provide rice breeders or food industries new opportunities to promote the production of rice or rice products with enhanced levels of the bioactive compounds.

2. Materials and methods

2.1. Rice samples

Fifteen rice (*Oryza sativa* L.) genotypes, which consisted of 5 non-pigmented brown rice (9311, Nipponbare, Tianyouhuazhan, II You 838, Yanfeng 47), 5 red rice (Jinggangshanhongmi, Xiangwanxian 12, Qianxiuhong, Changhong No. 1, Hongmi 2), and 5 black rice (D Youzinuo 161, Heimi No. 1, Heixiangnuo No. 3, Heinuomi, Heimi 2420) were selected for the study. Yanfeng 47 was planted in 2015 in the saline and alkaline land of Jinan, Shangdong province, China. All the other genotypes were grown in 2015 at a farm belonging to the China National Rice Research Institute located in Hangzhou, China. After maturing, the grains were harvested, sun-dried to a moisture content of about 13%, stored in an air-tight plastic bag at room temperature for three months, and then stored at 4 °C in the dark before analysis. The rough rice samples were de-hulled on a Satake Rice Machine (Satake, Tokyo, Japan) and milled to pass through a 100-mesh sieve on a cyclone sample mill (Foss, Switzerland).

2.2. Chemicals

Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azino-bis-(3-ethylbenzonthiazoline-6-sulfonic acid) diammonium salt) and gallic acid, catechin, vanillin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium hydroxide, sodium nitrite, aluminum chloride hexahydrate, potassium preoxydisulfate, hydrochloric acid, sulfuric acid and sodium sulfate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The HPLC grade methanol and ethyl acetate, used in the extraction, purification and HPLC analysis were purchased from Merck (Darmstadt, Germany) and Tedia (Fairfield, OH, USA), respectively. The standards of gallic, protocatechuic, *2,5*-dihydroxybenzoic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic, isoferulic, *o*-coumaric acid, cyanindin-3-*O*-glucoside, peonidin-3-*O*-glucoside were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). HPLC grade acetic and formic acid was purchased from Macklin (Shanghai, China). The certified mineral standards in rice flour samples (GBW 10010, CRM Rice) were obtained from the National Institute Center of Standards (Beijing, China).

2.3. Determination of color parameters

Color parameters of all samples were measured by the color difference meter (Konica Minolta, Japan), and performed in triplicate. They were expressed as tristimulus parameters, L^*, a^* and b^*. L^* indicates lightness (100 = white, 0 = black); a^* indicates redness-greenness (positive = red); b^* indicates yellowness-blueness (positive = yellow). In addition, the chroma (C) value which indicates color intensity or saturation was calculated as $C = (a^{*2} + b^{*2})^{1/2}$, and hue angle was calculated as $H° = \tan^{-1}(b^*/a^*)$.

2.4. Extraction of soluble-free, soluble-conjugated and insoluble-bound phenolics

Soluble-free, soluble-conjugated, and insoluble-bound phenolics were extracted according the method reported by Shao et al. (2014a). Briefly, soluble phenolics of rice flour were extracted with 80% methanol twice. The mixture was centrifuged (Himac CR21GⅡ, Hitachi, Japan) at 10,000*g* for 15 min at 4 °C. The soluble phenolics were concentrated by a rotary evaporator (IKA RV10, German) at 37 °C to get concentrated soluble phenolics. In order to get soluble-free phenolics, the concentrated soluble phenolics were extracted by ethyl acetate three times, and then dried by rotary evaporator, and dissolved in 5 mL of 50% methanol. To get soluble-conjugated phenolics, the concentrated soluble phenolics were hydrolyzed using 4 M NaOH for 2 h followed by adjusting pH to 1.5–2.0, extraction with ethyl acetate, drying using a rotary evaporator, and then dissolving in 5 mL of 50% methanol. After the extraction of soluble phenolics, the residues were used to extract insoluble-bound phenolics. The protocols were similar to the extraction of soluble-conjugated phenolics from concentrated soluble phenolics extracts by using 4 M NaOH and ethyl acetate. All extractions were performed in triplicate.

2.5. Determination of total phenilc content (TPC)

TPC was measured by Folin-Ciocalteu assay as reported by Shen et al. (2009). The results were expressed as milligrams of gallic acid equivalent per 100 gram of dry rice flour (mg GAE/ 100 g). Each extract was measured in duplicate.

2.6. Determination of ABTS radical scavenging activity (ABTS)

Total antioxidant activity of ABTS radical scavenging was assayed using spectrophotometry (Shen et al., 2009). The results were expressed as micromoles of trolox equivalent antioxidant activity per gram of dry rice flour (μM TE/g). Each extract was measured in duplicate.

2.7. Determination of DPPH radical scavenging activity (DPPH)

DPPH radical scavenging activity was carried out according to Beta, Nam, Dexter, and Sapirstein (2005). Total antioxidant activity of DPPH radical scavenging was expressed as micromoles of trolox equivalent antioxidant activity per gram of dry rice flour (μM TE/g). Each extract was measured in duplicate.

2.8. Determination of total flavanoid content (TFC)

TFC was assayed using a colorimetric method as reported by Shen et al. (2009). The results were expressed as milligrams of catechin equivalent per 100 g of dry rice flour (mg CE/100 g). Each extract was measured in duplicate.

2.9. Determination of total proanthocyanidin content (TPAC)

TPAC was determined using the vanillin assay (Sun, Ricardo-da-Silva, & Spranger, 1998). The results were expressed as milligrams of catechin equivalent per 100 gram of dry rice flour (mg CE/100 g). Each extract was measured in duplicate.